

Claims

1. Isolated, purified embryonic stem cell-derived precursor cells with neuronal and glial properties, wherein said precursor cells are non-tumorigenic after transplantation into the nervous system.

5 Sub B3 2. Isolated, purified and non-tumorigenic precursor cells with neuronal and glial properties, obtained from embryonic stem cells and containing no more than 15% primitive embryonic and non-neuronal cells, obtainable by

- (a) proliferation of ES cells,
- (b) cultivation of the ES cells from (a) into neural precursor cells,
- (c) proliferation of the neural precursor cells in growth factor-containing serum-free medium,
- (d) proliferation of the neural precursor cells from (c) in another growth factor-containing serum-free medium and isolation of the purified neural precursor cells and
- (e) proliferation of the neural precursor cells from (d) in another growth factor-containing serum-free medium and isolation of the purified precursor cells with neuronal or glial properties.

3. Cells according to claim 2, wherein the ES-cells in (a) are proliferated to cell aggregates, particularly embryoid bodies.

25 4. Isolated, purified and non-tumorigenic precursor cells with neuronal and glial properties, obtained from embryonic stem cells, containing no more than 15% primitive embryonic and non-neuronal cells, obtainable by

- (a') proliferation of ES cells,
- (b') cultivation of the ES cells from (a') into neural precursor cells,
- (c') proliferation of the neural precursor cells in growth factor-containing serum-free medium,
- (d') proliferation of the neural precursor cells from (c') in another growth factor-containing serum-free medium to neural spheres and isolation of the neural spheres and
- (e') proliferation of the neural spheres from (d') in growth factor-containing medium until generation of a monolayer of glial precursor cells and isolation of the purified precursor cells with glial properties.

5. Cells according to claim 4, wherein the ES-cells in (a') are proliferated to cell aggregates, particularly embryoid bodies.

6. Cells according to ^{claim 2} any one of claims 1 to 5 wherein said cells grow as monolayer.

7. Cells according to ^{claim 2} any one of claims 1 to 5, wherein said cells grow as neural spheres.

8. Cells according to ^{claim 2} any one of claims 1 to 5, comprising cells with neuronal, astroglial and/or oligodendroglial properties.

9. Cells according to ^{claim 2} any one of claims 1 to 5, wherein the ES cells were obtained after nuclear transfer into oocytes.

10. Cells according to ^{claim 2} any one of claims 1 to 5, wherein the ES cells were obtained from embryonic germ cells.

11. Cells according to ^{claim 2} any one of claims 1 to 5, wherein the cells are mammalian cells.

12. Cells according to claim 11, wherein the cells were isolated from the group comprising mouse, rat, hamster, pig, cow, primate and human.

13. Cells according to ^{claim 2} any one of claims 1 to 10, wherein the cells were genetically modified.

14. Cells according to ^{claim 2} any one of claims 1 to 13, wherein the cells are in a frozen condition.

15. Cell library comprising autologous and non-autologous cells according to ^{claim 1} any one of claims 1 to 14.

16. Neural spheres comprising neural cells differentiated from precursor cells according to ^{claim 1} any one of claims 1 to 5.

17. Neural spheres according to claim 16, obtained through in vitro differentiation of precursor cells as claimed in ^{claim 1} any one of claims 1 to 5.

18. A method for the generation of purified precursor cells with neuronal or glial properties, comprising:

5 (a) proliferation of ES cells,
(b) cultivation of the ES cells from (a) into neural precursor cells,
(c) proliferation of the neural precursor cells in growth factor-containing serum-free medium,
(d) proliferation of the neural precursor cells from (c) in another growth factor-containing serum-free medium and isolation of the purified neural precursor cells and
(e) proliferation of the neural precursor cells from (d) in another growth factor-containing serum-free medium and isolation of the purified precursor cells with neuronal or glial properties.

10 19. Method according to claim 18, wherein the ES cells in (a) are proliferated to cell aggregates, particularly embryoid bodies.

15 20. Method according to ^{claim 18} claim 18 or claim 19, wherein the growth factor-containing, serum-free medium in (c) comprises bFGF.

20 21. Method according to ^{claim 18} any one of claims 18 to 20, wherein the growth factor-containing, serum-free medium in (d) comprises bFGF and EGF.

25 22. Method according to ^{claim 18} any one of claims 18 to 21, wherein the growth factor-containing, serum-free medium in (e) comprises bFGF and PDGF.

23. Method for the generation of purified precursor cells with neuronal or glial properties, comprising:

30 (a') proliferation of ES cells ,
(b') cultivation of the ES cells from (a') into neural precursor cells,
(c') proliferation of the neural precursor cells in growth factor-containing serum-free medium,
(d') proliferation of the neural precursor cells from (c') in another growth factor-containing serum-free medium to spheres with neuronal and glial differentiation potential and isolation of the neural spheres and
(e') proliferation of the neural spheres from (d') in growth factor-containing serum-free medium until generation of a monolayer of glial precursor cells and isolation of the purified precursor cells with glial properties.

24. Method according to claim 23, wherein the ES-cells in (a') are proliferated to cell aggregates, particularly embryoid bodies.

25. Method according to ~~claim 23 or 24~~, further comprising the step
 (f') Manipulation of the differentiation of the glial precursor cells from step e') towards an astrocytic or an oligodendroglial fate and isolation of the precursor cells with astrocytic or oligodendroglial properties.

26. Method according to ~~any one of claims 23 to 25~~, wherein the growth factor-containing, serum-free medium in (c') comprises bFGF.

27. Method according to ~~any one of claims 23 to 26~~, wherein growth factor-containing, serum-free medium in (d'), (e') and (f') comprises bFGF and/or EGF.

28. Method according to ~~any one of claims 25 to 27~~, wherein the medium in (f') comprises either CNTF or T3.

29. Method according to ~~any one of claims 18 to 28~~, wherein the procedure is combined with cell separation and cell sorting techniques.

30. Method according to ~~any one of claims 18 to 29~~, wherein the purified precursor cells are suspended in a medium suitable for injection.

31. Use of the precursor cells, according to any one of claims 1 to 15, for the therapy of neural defects.

32. Use of the precursor cells, according to one of the claims 1 to 15, for the reconstitution of neuronal cells.

33. Use of the precursor cells, according to any one of claims 1 to 15, for the remyelination of demyelinated areas of the nervous system.

34. Use of the precursor cells, according to one of the claims 1 to 15, for cell-mediated gene transfer into the nervous system.

35. Use of the precursor cells, according to any one of claims 1 to 15, for the in vitro generation of polypeptides.

36. Use of the precursor cells, according to any one of claims 1 to 15, for transplantation into the nervous system.

5 37. Use of the neural spheres, according to claim 16 or claim 17, for transplantation into the nervous system.

10 38. A pharmaceutical composition containing precursor cells according to any one of claims 1 to 15 for the therapy of neural defects.

add A² ↗ add B⁵ ↗